Standard Operating Procedure for the Analysis of Nitrogen in Nitrate or Nitrite Form

1.0 Scope and Applicability

This method is applicable to the determination of nitrite alone or nitrite plus nitrate in surface and saline waters, domestic and industrial wastes. All standard curves are linear. The ranges are 0.003 to 1 ppm for Nitrite and 0.01 to 100 ppm for Nitrate plus Nitrite. This range may be extended by dilution if needed.

2.0 Summary of Method

NO_3^- is reduced to NO_2 almost quantitatively in the presence of cadmium. This method uses commercially available Cd granules treated with copper sulfate and packed in a glass column.

The NO_2^- produced thus is determined by diazotizing with sulfanilamide and coupling with N- (1-naphthyl)-ethylenediamine dihydrochloride to form a highly colored azo dye that is measured colorimetrically at 520 µm on the FIA. The normal range for this procedure is up to 10 mg Nitrate plus Nitrite /L. This procedure has been modified to directly read up to 100 mg N/L by using a 0.1 cm path length flow cell on channel two. This provides an on line optical dilution of 1:10.

3.0 Definitions

The definitions and purposes below are specific to this method, but have been conformed to common usage as much as possible.

FIA	Flow injection analysis (a colorimetric procedure)
mg N/L	milligrams Nitrogen per Liter
ppm	parts per million
MDL	Method Detection Level
PDL	Performance Detection Level
LIMS	Laboratory Information Management System

4.0 Interferences

Samples to be run should be free of suspended matter as this will hang up in the column and shift window timing. Concentrations of iron, copper or other metals above several milligrams per liter lower reduction efficiency. Oil and grease will coat the Cd surface. If present try to avoid including this in the sample. Residual chlorine will oxidize the column and shorten its life. Residual chlorine can be removed with sodium thiosulfate ($Na_2S_2O_3$) solution 3.5 g/L, but residual chlorine removal is not practiced. See column precautions on sodium thiosulfate. Colored samples which absorb at 520 nm interfere.

From time to time samples for nitrate analysis have been found to produce negative peaks. In most runs the ammonium chloride buffer made from NH_4Cl and NaOH tends to produce

a slight pink background color, after passing over the cadmium column, suggesting NO_3 contamination. Ammonium chloride buffer made from NH_4OH and HCl does not have a pink color after passing through the cadmium column.
In one comparison between "problem" samples, a problem sample was found to have a residual chlorine similar to BOD samples as opposed to the normal sample of similar matrix and storage conditions which had no residual chlorine.

It is theorized that a trace amount of nitrate in either or both the NH_4Cl or the sodium hydroxide tend to elevate the baseline. This baseline is then reduced producing negative peaks when a problem sample is encountered which contains residual chlorine. The residual chlorine bleaches or reduces the intensity of the background color. Although this may explain the problems we had with a few safe drinking water samples it does not explain the interference encountered is a batch or two of class R samples received in the spring of 1993. The alternate recipe for ammonium chloride buffer is provided as a solution to the negative peak problem.

5.0 Safety:

WARNING This method uses Cadmium granules. Cadmium is toxic and carcinogenic. Wear latex gloves when copperizing the granules. All waste Cadmium will be retained in a 200 ml waste bottle and held until such time as a contract is issued for waste disposal for the whole lab.
This method does not address all safety issues associated with its use. The laboratory is responsible for maintaining a safe work environment and a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material safety data sheets (MSDS's) should be available to all personnel involved in these analyses.

6.0 Equipment and Supplies:

Note: Brand names, suppliers, and part numbers are cited for illustrative purposes only. No endorsement is implied. Equivalent performance may be achieved using equipment and materials other than those specified here, but demonstration of equivalent performance that meets the requirements of this method is the responsibility of the laboratory.

6.1 Lachat QuikChem 8000 Automatic Flow Injection Analyzer with:

6.1.1 AIM 1250 XYZ sampler

6.1.2 Ismatec 12 position proportioning pump

6.1.3 injection module

6.1.4 two channel colorimeter

6.1.5 10 mm path flow cell

6.1.6 1 mm path flow cell

6.1.7 520 nm interference filters (2)

6.1.8 18 cm sample loop

6.1.9 Cadmium column (150 X 3 mm glass column sold by Chrom Tech, Apple Valley, MN. as part # OM-6312)

6.1.11 Gateway E-4200 computer with omnion 2.0 software

6.1.12 Gateway EV700 monitor

6.1.13 HP 8150 printer

7.0 Reagents and Standards:

All reagents are ACS Reagent grade or higher.

Reagents:

7.1 Ammonium chloride buffer:

Dissolve 170 g of ammonium chloride (NH_4Cl) and 2.0 g of disodium ethylenediamine tetracetate dihydrate (EDTA), ($NaC_{10}H_{14}O_8N_2 \cdot 2H_2O$) in approximately 1600 mL of distilled water. Add 40 mL of 10 N NaOH and dilute to 2 liters. The pH should be about 8.5. Degas with helium.

7.2 Ammonium chloride buffer (alternative recipe):

To 500 mL of deionized water in a 2 L beaker add 210 mL of concentrated HCl, 190 mL of concentrated ammonium hydroxide (NH_4OH) and 2.0 g of EDTA. Dissolve all of the above and bring the solution to a volume of 2 L.

7.3 Color Reagent:

To approximately 1600 mL of distilled water add 200 mL of conc. H_3PO_4. Add 80 g of sulfanilamide and dissolve completely. Dissolve 2.0 g N-1-Naphthylenediamine dihydrochloride ($C_{10}H_7NHCH_2CH_2CH_2 \cdot 2HCl$) dilute to two liters. Store in a dark bottle. This reagent is stable for one month. Degas with helium.

7.4 Granulated Cadmium:
40-60 mesh (E.M. Laboratories, Inc. 500 Exec. Blvd. Elmsford, NY 10523, Cat 2001-2 Cadmium, Coarse Powder).

7.5 Line cleaning solution:
Dissolve 65 g sodium hydroxide (NaOH) in 800 mL deionized water. Add 6 g EDTA and dilute to 1 liter. Other solutions such as 1:1 HCl or Contrad 70 (Fisher Scientific, Pittsburgh Pa. (Cat.# 04-355-1)) have also been found to clean well.

7.6 Dilute hydrochloric acid: (6 N)
Dilute 50 mL concentrated HCl to 100 mL with distilled water.

7.7 Copper sulfate solution 2%
Dissolve 20 g of ($CuSO_4 \cdot 5\ H_2O$) in 500 mL of distilled water and dilute to 1 liter.

Standards:

7.8 Stock Nitrite Standard (100 ppm N/L as NO_2).
Dissolve 0.4926 g of sodium nitrite ($NaNO_2$) Reagent grade chemical, in approximately 800 mL distilled water and dilute to 1 liter. Prepare the nitrite stock solution fresh weekly as it is not stable.

7.9 Stock Nitrate Standard (1000 ppm N/L as NO_3)
Dry potassium nitrate (KNO_3), (Fisher Reagent Grade, for 24 hours at 105 ºC. Store in a desiccator until ready for use. Dissolve 7.2674 g KNO_3 containing 13.76% N or equivalent nitrate standard) in 800 mL distilled water. Add 2 mL chloroform and bring to volume. Store in a dark bottle. The reagent is stable for 6 months.

7.10 Spike Solutions
Nitrate spike solution (100 ppm N as NO_3)
Dissolve 0.7267 g KNO_3 in about 800 mL of deionized water. Add 2 mL chloroform and bring to volume. Store in a dark bottle. The reagent is stable for six months.

7.11 Nitrite spike solution (100 ppm N as NO_2)
Use the nitrite standard prepared fresh weekly.

7.12 Working Nitrate Standard (100 ppm N/L as NO_3)
Pipette 20 ml of the stock nitrate standard (8.9) into a 200 ml volumetric flask, and bring to final volume with deionized water. Invert the flask ten times to mix and store in a 200 ml plastic bottle in the refrigerator. Replace monthly.

Calibration Standards:

7.13 **Nitrite** standards are prepared daily by diluting the 100 mg nitrite/L solution (8.8) into a 100 ml volumetric flask according to the following table. The nitrite samples are run on the Quikchem 8000 without the cadmium reduction column. The reagents are the same.

level	ml used	mg Nitrite/L
7	2	2.000
6	1	1.000
5	0.5	0.500
4	0.25	0.25
3	0.1	0.100
2	0.05	0.05
1	0	0.000

7.14 **Nitrate** Standards are prepared from the 100 ppm working nitrate standard (8.12) by making the following dilutions into 100 ml volumetric flask.

Level	**ml used**	**mg Nitrate/L**
8	all stock standard	100
7	50	50
6	10	10
5	5	5
4	1	1
3	0.5	0.5
2	0.05	0.05
1	0	0

All Standards are placed into the autosampler. The software will use level 1 to 8 excluding #2 for the High level calibration running on channel #2, and will use levels 1 thru 6 for the low level analysis running on channel #1 of the QuikChem 8000.

8.0 Sample Collection, Preservation and Storage

Nitrate samples not analyzed within 48 hours should be preserved with H_2SO_4 and cooled to 4 ºC. The holding time is 28 days. Nitrite samples have a holding time of 48 hours from the time of collection. It is the responsibility of the login staff to place the nitrite samples into the small refrigerator, place the "contains nitrite" sign on the front of the refrigerator and notify the analyst that a nitrite sample has arrived. Login staff, and those making scheduling arrangements for samples, should attempt to have nitrite samples arrive in the lab the first part of the week due to the short holding time.

CAUTION: No samples preserved with mercuric chloride should be run on the column as they will degrade the cadmium column.

9.0 Quality Control

Each set of 10 samples or less will include 1 check sample, 1 duplicate and 1 spiked sample. The software performs a least square regression on the standards. The correlation coefficient for this regression must be 0.995 or higher. Improvement may be made by omitting an outlier. If improvement is not made the standards should be rerun. If this fails to improve the regression then new standards should be prepared. Duplicate, spike and check samples must be within acceptable limits. Each set of samples will include a laboratory fortified blank which must run within $\pm$ 10% of its true value.

Following standardization for (nitrate plus nitrite), an analysis for a nitrite and nitrate standard of equal concentration (1 mg N/L) will be run. This ratio should show 80% or better on conversion of nitrate to nitrite. Below this level the Cadmium column should be exchanged with a freshly packed one. The column efficiency is not in itself a direct measure of the % of nitrate being measured, as the standards will also show the same conversion loss as the samples. Samples which contain a large amount of nitrite will tend to yield higher than correct results. If high levels of nitrite are suspected then the actual amount of nitrite should be measured and a calculation performed to correct this estimate of the true nitrate plus nitrite. See section 18.0 for an example calculation.

10.0 Calibration and Standardization:

Standardization is achieved using Lachat's Omnion software ver 2.0. A Typical calibration curve is displayed in section 18.0. The number of standards and their levels are listed in section 8.13 and 8.14. All Standards curves are linear and will run with a correlation coefficient of 0.995 or better for Nitrate + Nitrite analysis and 0.999 or better for Nitrite analysis. Failure at this point will require a re-standardization or preparation of new standards. All data stored with the Omnion software used on the QuikChem 8000 provides for imbedding of the calibration curve in the data file. The electronic data file including the chromatograms can be retrieved and reviewed. The file can be reprocessed with new parameters but the original data will remain unchanged.

11.0 Procedures

11.1 Automated Colorimetric Procedure

Setting up the Quik-Chem 8000 FIA for the Nitrate Chemistry (nitrate + nitrite)

11.1.1 Place two 520 nm filters into the colorimeter on channels one and two. Install the 18 cm sample loop on channel #1. Place a 0.1 cm flow cell into the detector of channel two. Run the waste line from channel one to the inlet of channel two. Run waste line from channel two to waste. Install the Nitrate manifold board on channel one. Run feed lines through the pump cassette, connect pump tubes to the manifold according to the diagram (Section 18.0), and snap the pump tubes into place. Turn on the pump. Place all feed lines into deionized water. Check for leaks.

11.1.2 Place the Cd column in the circuit and put all feed lines into their proper reagent containers. Run until all reagents have filled the manifold board..

11.1.3 When samples are loaded into the autosampler load the proper method on the computer: “nitrate.met". Load the tray “nitrate.tra”. Start the analysis.

11.1.4 Check the Standardization and the column efficiency before continuing the run. See section 10.0.

11.2 Cadmium Preparation

WARNING: Cadmium is toxic and carcinogenic. Wear latex gloves when copperizing the granules. The cadmium granules are cleaned with dilute 6N HCl and copperized with a 2% solution of copper sulfate as follows:

11.2.1 Wash the cadmium with HCl and rinse with distilled water. The color of the cadmium so treated should be silver.

11.2.2 Swirl 10-20 g of cadmium in 100 mL portions of 2% solution of copper sulfate for five minutes or until the blue color partially fades, decant, and repeat with fresh copper sulfate until a gray or black appearance. A brown colloidal precipitate indicates to much copper sulfate. Rinse to remove.

11.2.3 Wash the cadmium-copper with ammonium chloride solution several times to remove all the precipitated copper. The color of the cadmium so treated should be grey to black.

11.3 Re-packing the Cadmium Column

WARNING: Cadmium is toxic and carcinogenic. Wear latex gloves when repacking the column(s). After the column efficiency has dropped below 80% the column should be re-packed.

11.3.1 Open up both ends of the column, remove the end plugs. Hold the glass column over the waste cadmium container and gently tap and/or rinse the granules into the waste bottle with a stream of distilled water.

11.3.2 Rinse the column with distilled water. Replace one polyurethane foam plug and connect one plastic end with teflon tubing. Connect a short piece of tygon tubing between the threaded end of the column and a thistle tube bowl or small funnel.

11.3.3 Fill the thistle tube with ammonium chloride solution. Using a pipped bulb without the pipped holder, force the ammonium chloride down the column and out the TEFLON tube.

11.3.4 Add the copperized cadmium granules prepared earlier a few at a time to the thistle tube with a spatula and tap gently to solidify the cadmium in the column.

11.3.5 Remove the tygon tubing from the top and insert a foam plug. Re-attach the other end of the column with its threaded counterpart using teflon tape. Store until ready for use. When re-tightening the threaded plastic ends to the glass column check to be sure all threads are free of small pieces of cadmium or chipping can occur during tightening resulting in leaks.

11.3.6 The column must be activated before use. Running the standards through usually will do it. If standards fail the first time, re-standardize.

11.4 Regeneration of the Cadmium column

The life of the column can be extended by gently running about 2-5 ml of the 2% copper sulfate soln thru the column when off line by using a syringe.

12.0 Data Analysis:

Calculations for this procedure are performed by the FIA computer using a least squares linear regression on the standards. Results will be reported in mg N/L to three decimal places. Calculations involving the efficiency of the columns, when needed, are shown in Section 18.0.

13.0 Method Performance:

The following method detection levels (MDL) and precision were obtained by spiking ten samples of deionized water with 0.005 ppm nitrite and 0.01 ppm nitrate standards. The MDL's are compared to the Performance Detection Level (PDL) obtained from our data base using 38 duplicates for nitrate over the range of 0.02 to 0.06 ppm. There is insufficient data for a determination of actual performance on nitrite samples.

Analyte#	Analyte	PDL	precision(σ)	MDL	precision(σ)
9570	nitrite			0.000401	.000159
9555	nitrate+nitrite	0.0198	0.00816	0.00511	.002004

14.0 Pollution Prevention:

A major source of pollution in this method is the use of Cadmium. All Cadmium after use will be collected and stored until a safe disposal plan becomes available.

15.0 Waste Management:

Due to the hazardous nature of Cadmium its use should be minimized as much as possible. One way to do this is to use the prepared columns and try to regenerate them as many times as possible before discarding the waste into storage bottles. See Section 12.4.

For further information on waste management consult The Waste Management Manual for Laboratory Personnel and Less is Better: Laboratory Chemical Management for Waste Reduction, both available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th Street N.W., Washington, D.C. 20036.

16.0 References

16.1 EPA (August 1993) Method 353.2 Revision 2.0 (Determination of Nitrate-Nitrite by Automated Colorimetry)

16.2 Lachat QuikChem Method No. 10-107-04-1-C

16.3 EPA Appendix B to Part 136 - Definition and Procedure for the Determination of the Method Detection Limit - Revision 1.11, 40 CFR Ch.1 d(7-1-94 Edition).

16.4 1030C. Precision using duplicates, *Standard Methods for the Examination of Water and Wastewater,* 18th ed. 1992, American Public Health Association, 1015 fifteenth St. NW Washington, DC 20005

17.0 Tables, Diagrams, Flowcharts, Validation Data and Additional Information

Raw data to be saved includes the printout of the mg N/L for the samples along with the printing of the regression analysis of the standards. After the data has been entered into the LIMS, the distribution sheet will also be kept. Completed results will be kept in either the High level nitrate (9555), or nitrite (9570) book (s) according to date of analysis.

Correction for efficiency of Cadmium column.

In the normal real samples the efficiency of the column will distort the standards and the samples equally and our nitrate + nitrite values are very good because of the small amount of nitrite. However if the nitrite contribution is substantial then all of the nitrite is being measured against a nitrate standard which is lower than it should be by the efficiency of the column. The result is that the total value of Nitrate + nitrite is over inflated. To correct this the following calculation will need to be made.

NO_3 present = $(NO_3 + NO_2)$ - $(NO_2$/column efficiency)

Example: A sample ran with a value of 5.87 mg N/L for nitrate + nitrite. The nitrite value of this sample was 1.40 mg N/L. The 1 ppm nitrite and 1 ppm nitrate ran 1.27 and 1.01 ppm respectively.

The column efficiency was 1.01/1.27 = 0.795

The true nitrate value was 5.87 - (1.40/0.795) = 5.87 - 1.76 = 4.11

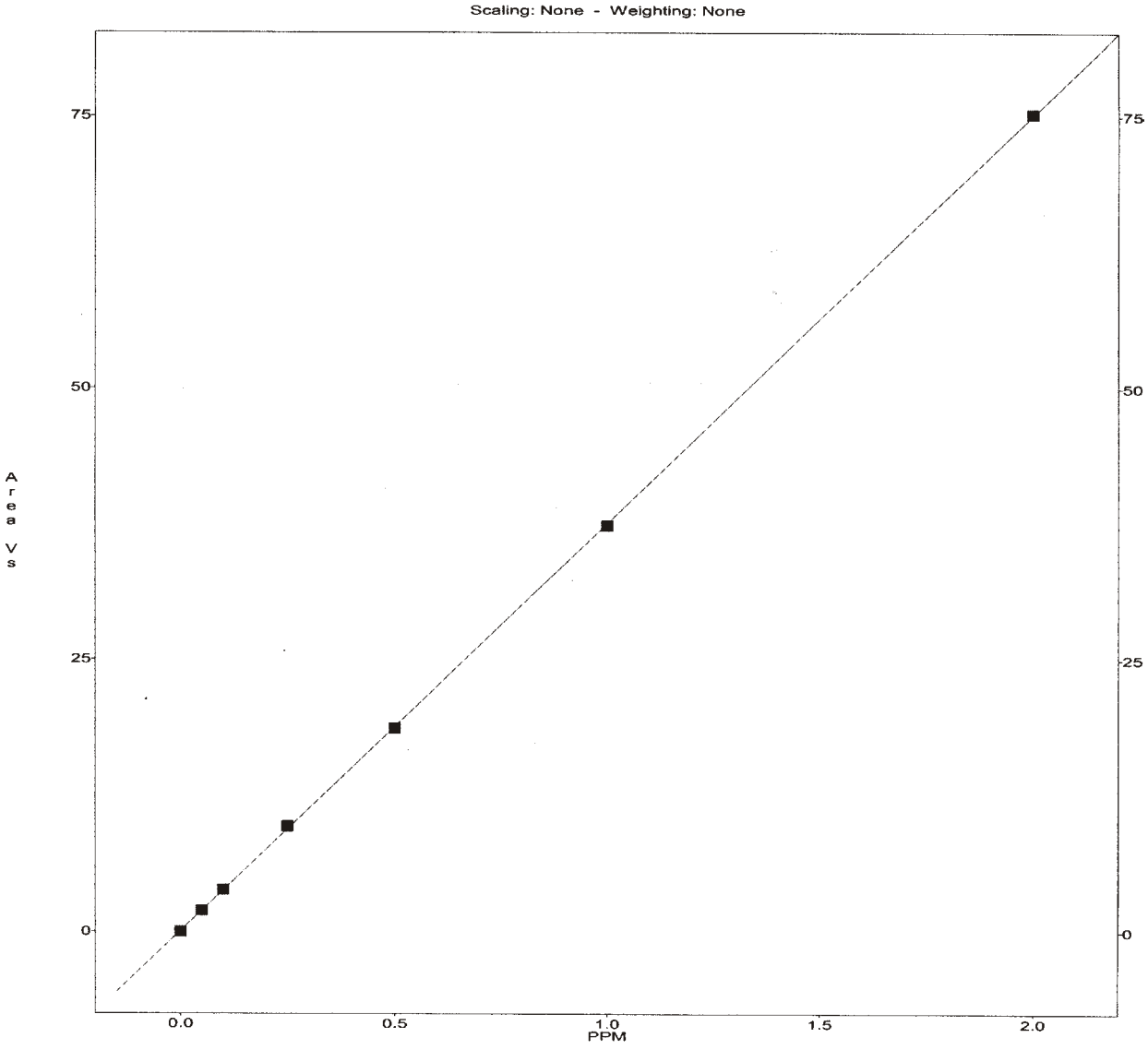
The corrected nitrate + nitrite value is then:

4.11 nitrate + 1.4 nitrite = 5.51 mg nitrate + nitrite /L

Nitrite

Lvl	Area	PPM	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Replic STD	Replic % RSD	Residual 1st Poly
1	75229192	2.00	75229192					0.0	0.0	-0.1
2	37390828	1.00	37390828					0.0	0.0	0.6
3	18698540	0.50	18698540					0.0	0.0	0.8
4	9710651	0.25	9710651					0.0	0.0	-2.7
5	3864882	0.10	3864882					0.0	0.0	-0.9
6	1967142	0.05	1967142					0.0	0.0	-0.7
7	24918	0.00	24918					0.0	0.0	

1st Order Poly
Conc = 2.665e-008 Area - 2.079e-003
r = 1.0000

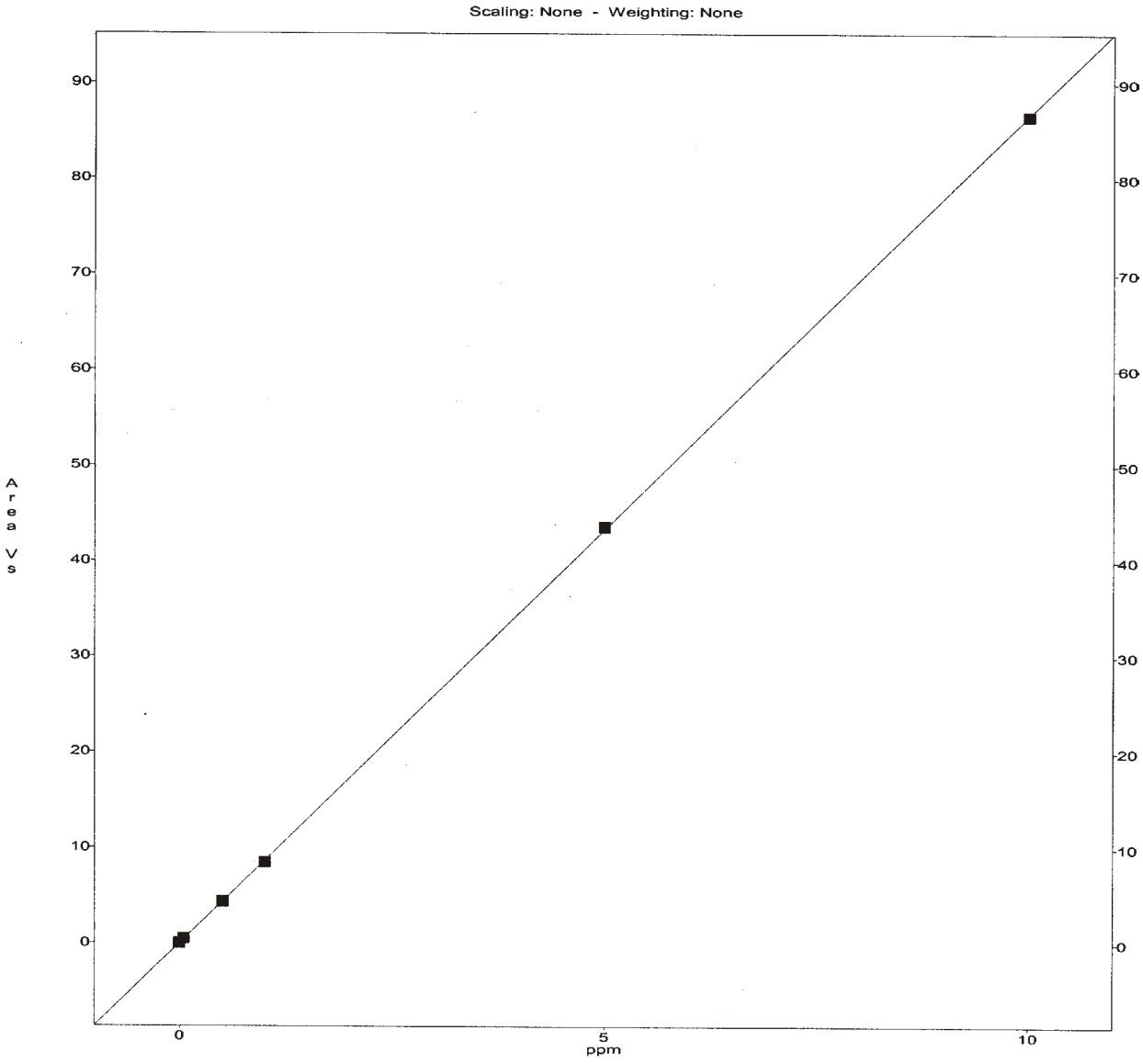


Printed: Tuesday, March 11, 2003 - 11:30 AM

Low nitrate

Lvl	Area	ppm	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Replic STD	Replic % RSD	Residual 1st Poly
3	86578152	10.00	86578152					0.0	0.0	0.1
4	43610080	5.00	43610080					0.0	0.0	-0.6
5	8465779	1.00	8465779					0.0	0.0	2.3
6	4367569	0.50	4367569					0.0	0.0	-0.8
7	493040	0.05	493040					0.0	0.0	-13.7
8	27183	0.00	27183					0.0	0.0	

1st Order Poly
Conc = 1.153e-007 Area + 0.000e+000
r = 1.0000

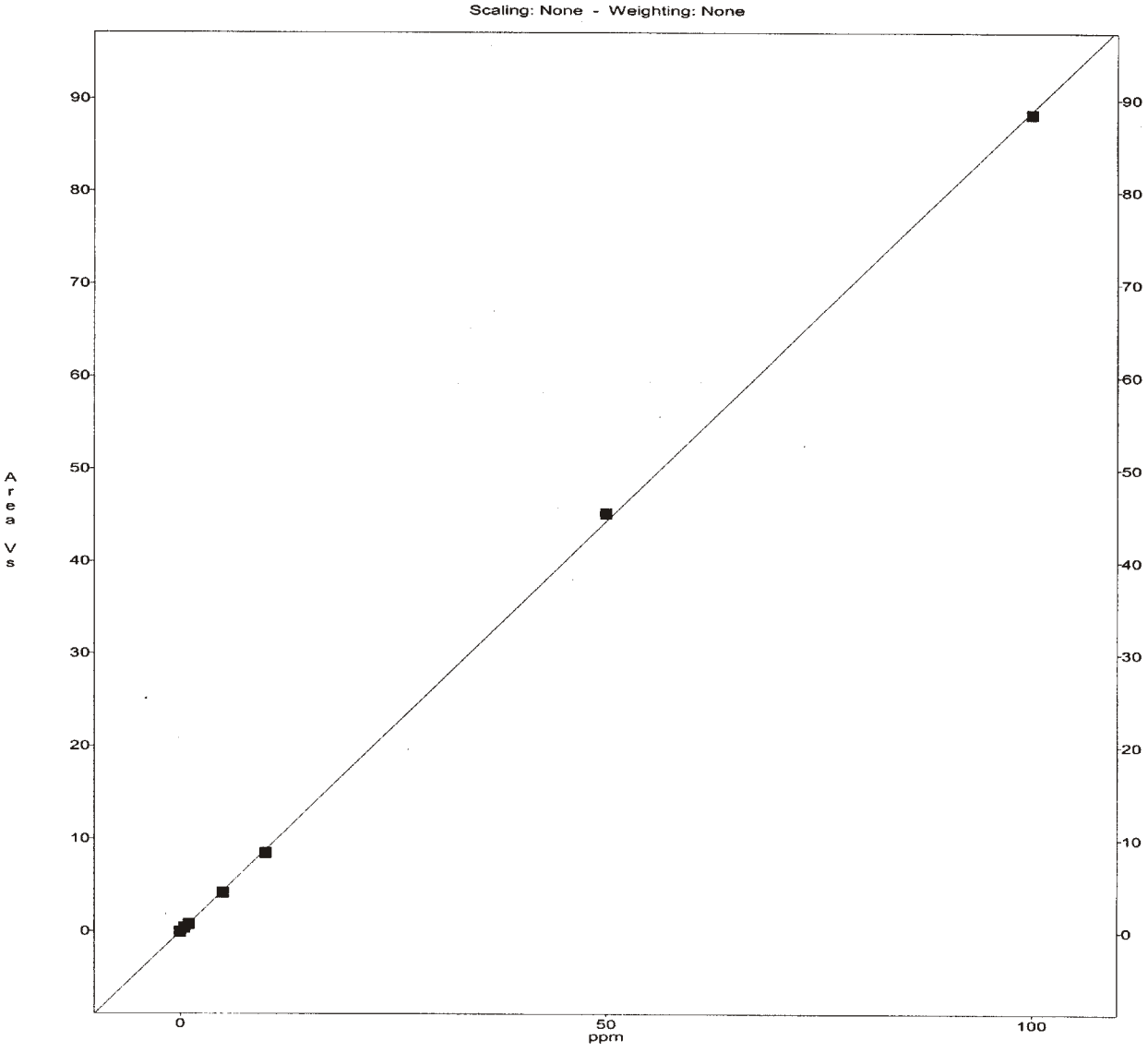


Printed: Tuesday, March 11, 2003 - 10:03 AM

High nitrate

Lvl	Area	ppm	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Replic STD	Replic % RSD	Residual 1st Poly
1	88409480	100.0	88409480					0.0	0.0	0.4
2	45202396	50.0	45202396					0.0	0.0	-1.9
3	8532192	10.0	8532192					0.0	0.0	3.5
4	4230970	5.0	4230970					0.0	0.0	3.7
5	812730	1.0	812730					0.0	0.0	3.5
6	417613	0.5	417613					0.0	0.0	-4.0
8	0	0.0	0					0.0	0.0	

1st Order Poly
Conc = 1.126e-006 Area + 5.016e-002
r = 0.9999



Printed: Tuesday, March 11, 2003 - 10:03 AM

OPERATOR: dennis
ACQ. TIME: Dec 20, 1995 14:33:02
DATA FILENAME: C:\OMNION\DATA\9512200C.FDT
METHOD FILENAME: C:\OMNION\METHODS\NO3.met
TRAY FILENAME: C:\OMNION\TRAYS\NO3.tra
TRAY DESCRIPTION:
1L nitrate

{95-5}

Multi-Channel Table
Type: Unknowns
Channel Range: 1 to 1 -- Cup Range: 1 to 40

Cup	Log Number	# of Reps	nitrate (PPM)	Man Dil Factor	Auto Dil Factor
1	kv:HLCHECK	1	39.0927	20.0	1.00
2	1ppmNO2	1	1.0461	1.0	1.00
3	1ppmNO3	1	0.9772	1.0	1.00
4	kv:LFB1ppm	1	1.0160	1.0	1.00
5	.01 ppm	1	0.0271	1.0	1.00
6	.01	1	0.0202	1.0	1.00
7	.01	1	0.0192	1.0	1.00
8	.01	1	0.0196	1.0	1.00
9	.01	1	0.0181	1.0	1.00
10	.01	1	0.0225	1.0	1.00
11	.01	1	0.0188	1.0	1.00
12	.01	1	0.0201	1.0	1.00
13	.01	1	0.0201	1.0	1.00
14	.01	1	0.0199	1.0	1.00

$n = 10$

$\bar{x} = .02057$

$s = .002598$

$MDL = (.002598)(2.821)$
$= .00733$ mg/L

S/N ratio: $\frac{.02057}{.002598} = 7.9$

$F = \frac{(.002598)^2}{(.001134)^2} = 2.29$ $\quad F_{(9,9,.90)} = 2.44$ $\therefore$ var. are same.

$S_{pooled} = \left[\frac{9(.002598)^2 + 9(.001134)^2}{18}\right]^{1/2} = \left[\frac{(6.7496E-6) + 1.285796E-6}{2}\right]^{1/2}$

$= \sqrt{\frac{8.03556E-6}{2}} = .002004$

$MDL = (S_{pooled})(t_{18,.99})$
$= (.002004)(2.552)$
$= .00511$ mg $NO_3 + NO_2$ / L

Calculations for the MDL for Nitrate

Calculations for MDL for Nitrite

OPERATOR: dennis
ACQ TIME: Dec 20, 1995 16:17:16
DATA FILENAME: C:\OMNION\DATA\9512200E.FDT
METHOD FILENAME: C:\OMNION\METHODS\NITRITE.met
TRAY FILENAME: C:\OMNION\TRAYS\NITRITE.TRA
TRAY DESCRIPTION:
NITRITE

{95.70}

Multi-Channel Table
Type: Unknowns
Channel Range: 1 to 8 -- Cup Range: 1 to 40

Cup	Sample ID	# of Reps	Nitrite (ppm)	Man Dil Factor	Auto Dil Factor
1	.005 ppm	1	0.00742	1.0	1.00
2	.005	1	0.00696	1.0	1.00
3	.005	1	0.00708	1.0	1.00
4	.005	1	0.00691	1.0	1.00
5	.005	1	0.00696	1.0	1.00
6	.005	1	0.00684	1.0	1.00
7	.005	1	0.00716	1.0	1.00
8	.005	1	0.00680	1.0	1.00
9	.005	1	0.00712	1.0	1.00
10	.005	1	0.00694	1.0	1.00

$n = 10$

$\bar{X} = .007019$

$\sigma = .000182547 98$

$MDL = (.000182547)(2.821)$

$= .0005151$

$F_{(9,9,.90)} = 2.44$

$F = \frac{(.00018248)^2}{(.00013209)^2} = 1.908$

$S_{pooled} = \left[\frac{9(.00018248)^2 + 9(.00013209)^2}{18}\right]^{1/2} = \left[\frac{9(3.3298E-8) + (1.74477E-8)}{18}\right]^{1/2}$

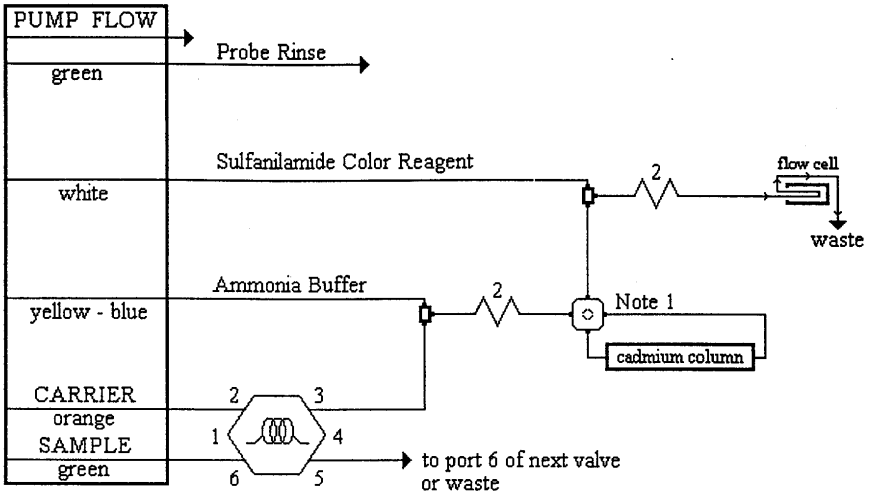
$= \sqrt{\frac{5.07457E-8}{2}}$

$= 1.5929E-4$

$MDL = (.00015929)(t_{18,.99})$

$= .0004014 \text{ mg } NO_3/L \quad (2.521)$

NITRATE/NITRITE, NITRITE MANIFOLD DIAGRAM:



Sample loop = 17 cm

Interference Filter = 520 nm